CLOSURE OF LONGITUDINALLY SPLIT TUBULARIAN STEMS.

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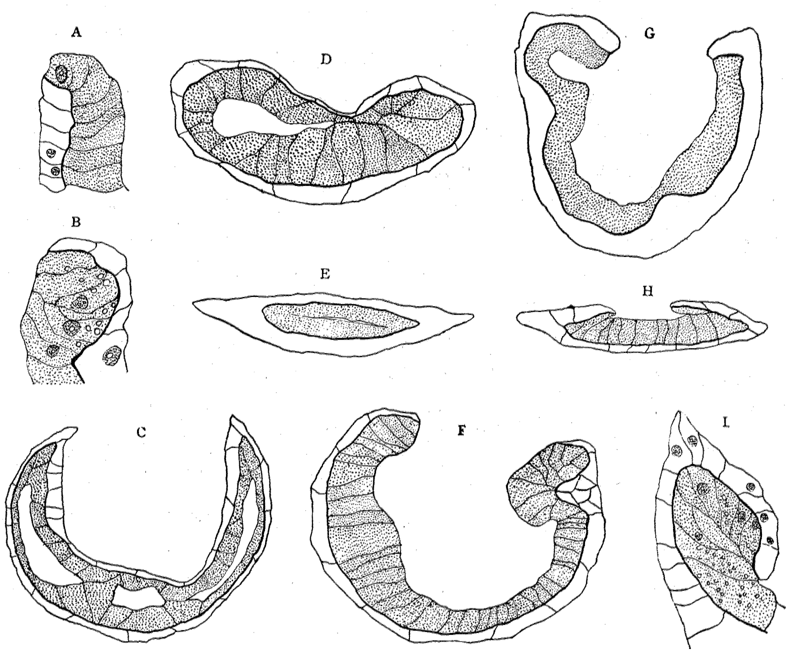
My work was carried on under the direction of Professor T. H. Morgan and Dr. N. M. Stevens, for whose suggestions and criticism I am deeply indebted.

The rapid closure of the cut edges of pieces of the stem of *Tubularia* which have been split longitudinally, has been studied by Morgan and by Godlewski. The latter has published the results of his observations in two recent papers. Godlewski worked with *Tubularia mesembryanthemum*, found at Naples, while my own work has been on *Tubularia crocea*, from Wood's Hole, Mass. The method of closing in this form appears to differ in a number of points from that of *Tubularia mesembryanthemum* described by Godlewski. Material that had been collected by Professor Morgan during the summer was sectioned and studied. The conclusions drawn from this work were then verified on fresh material, of which sections also were made. The study of the living material, however, is not very satisfactory, as it is possible to see so little of what is going on. The circulation of the fluid inside of the open tubes and the gradual stretching across of the membrane, are all that can be seen clearly.

The splitting was done with a pair of fine scissors. The material was killed and fixed in corrosive acetic. Somewhere in the process of preserving, probably in the alcohols, the cœnosarc shrinks and loosens from the perisarc, which keeps its former size and shape. This makes it difficult to study their relation to each other in the closing process. The tissues of the cœnosarc are well preserved by the corrosive acetic solution and can be studied easily. The sections were stained with Delafield's hæmatoxylin, followed by picric alcohol, according to the method of Stevens ('01). The picric alcohol stains the granules in the endoderm cells bright yellow, and thus makes the difference between ectoderm and endoderm cells distinct.

METHOD OF CLOSURE.

As the result of the splitting, the stems were divided into part-cylinders of various sizes, some almost complete, others with a mere strand of cœnosarc; while in some, an oblique split produced pieces nearly complete at one end and very small at the other. The method of closure seems to be independent of the size of the piece, the same general process being followed in all pieces. The cut leaves a free edge of both ectoderm and endoderm. In sections of pieces which had been killed five, ten or fifteen minutes after the splitting, the endoderm cells had rounded



up over the ectoderm, probably as a result of disturbing the osmotic pressures (Fig. A). The first step towards closure of the tube is the pushing of the ectoderm over the endoderm (Fig. B). Whether this is accompanied by any activity on the part of the endoderm cannot be stated definitely, but it seems probable from some sections and from what occurs in later stages, that the endoderm cells change their shape, their outer ends next the ectoderm becoming broader and the inner ends becoming pointed,

the cells thus taking on a triangular rather than a rectangular shape in section. This is shown in Fig. D. Some sections, however, do not show this at all, and we must conclude, that in some cases the endoderm lags a little behind the ectoderm.

During the subsequent process of closure, the ectoderm and the endoderm stretch across from both sides till they meet and form a two-layered membrane which closes in the digestive tract again. In this process of closing the two layers keep nearly even, but the ectoderm sometimes is a little farther advanced than the endoderm and curves around over its free end. The closing membrane does not arch outwards so as to complete the cylindrical form, nor does it grow straight across, which would be the shortest distance between the sides, but it is nearly always concave (Figs. C and D). In pieces which are much less than a half-cylinder, there is little chance for the membrane to bend in, and it lies close to the old wall (Fig. E). Also in cases where there is a large endodermal ridge, the bending in is either prevented or lessened.

The position of the endodermal ridges and their relation to the closure of the stem must now be considered. Godlewski has not taken these ridges into account, although one or two of his figures seem to indicate their presence. These ridges are found throughout the stem and cannot be ignored. Their presence causes some variations in the beginning of the process of closing and introduces certain conditions which might be easily misinterpreted. If the cut extends along the side of a ridge, or through it, the appearance in section of such a piece is very different from a section in which the ridge is not near the cut edge. There is a thick mass of cells at the edge, appearing somewhat like the beginning of a closing membrane (Fig. F). This mass is chiefly endoderm cells which contain many yellow-stained granules. If only a little of the ridge has been cut off, it is easily identified as a ridge by the thickening of ectoderm outside of it containing numerous dark stained nuclei; otherwise, identification is possible by tracing back through sections to a place where the cut was less deep and the ridge still intact. The ectoderm stretches over the cut endoderm until the two layers are even and then the two stretch across together and meet to form

a complete membrane. If the cut edge extends just above a ridge, when the membrane begins to stretch across, a cavity is left between the closing membrane and the ridge, which in some cases looks like a canal within the edge of the closing membrane, such as Godlewski describes (Fig. G). It may be that his canals are formed in this way and his figures might be so interpreted. In the sections of *Tubularia crocea*, I have found no canals formed by the disintegration of endodermal cells in the edge of the closing membrane. In the living material, no circulation has been observed within the edges of the closing membrane indicating the presence of canals, yet the circulation in the bottom of the open cylinder was easily seen, so that the circulation is not something peculiar to the canals of Godlewski.

The pieces may be cut off in such a way that a ridge is left in the middle of the half-cylinders. Sometimes it appears as though the growing edge of one side joined such a ridge, but this appearance was seen in but a few consecutive sections, showing that it would not have interfered with natural closing, in further development. If the piece is rather small, or the ridge large, the closing membrane is prevented from bending in as much as usual. But this middle ridge makes still greater complications in small pieces, as here it may fill up the cavity formed by the closing membrane, and cause an appearance of solidity.

In many of the smaller pieces, the sections, at first sight, look solid as though the endoderm cells had remained inactive, and lay in the same position that they were in when the piece was cut. It looks as though the ectoderm has grown over this solid mass, thus making a solid core of endoderm with a thin covering of ectoderm as Godlewski describes. But it is natural to expect that if the endoderm cells lag only a little behind the ectoderm cells in the closure of a large piece of a tubularian stem, they would do the same in a small piece of the same stem. If the work had been entirely with large and small pieces coming from different stems or different portions of the same stem, this expectation might not be justifiable, but the pieces used were obliquely split, large at one end and small at the other, the conditions for both ends thus being nearly alike. A contraction, if the closing is due to this, would be along the entire free edge regardless of

the size of the piece or the part of the tube, and if the endoderm and ectoderm both take part at one end, it is reasonable to expect that they would along the entire edge. With this idea in mind, the apparently solid closures were studied in order to see if there were not two layers of endoderm. The pieces were traced back through the series of sections to the place where less had been cut off in order to locate the ridges and in many pieces, a ridge was found included in the small piece. In some very small pieces, not including any ridge, it was found that the edges, both ectoderm and endoderm, had met to enclose a small but distinct cavity. Fig. H shows the section just before the edges meet. In other small pieces with no ridges the higher powers of the microscope showed two distinct layers of endoderm, indicating that the closure had been the same, but the piece was so flat and the angle turned by the closing membrane was so sharp that the cavity between the old and new walls was flattened out into a mere crack and the endoderm of the closing membrane practically touched the endoderm of the old wall (Fig. E). The presence of a ridge would cause the obliteration of the cavity in a piece otherwise large enough to leave a distinct cavity in closing. Fig. D shows this on the right side. The ridge is further toward the right, and consequently that side has no cavity. This section shows a circular arrangement of the endoderm cells at the point of turning, indicating a compression at their inner ends that would justify the statement that the layer of endoderm has been drawn around with the ectoderm.

In apparent contradiction to the above statement that only small pieces, or pieces including a ridge, close in solid, obscuring the cavity, a few sections complying with neither of these conditions show the ectoderm beginning to grow over the endoderm and leaving no cavity. But here, as in the small pieces there are two layers of endoderm in all cases where the ectoderm has overgrown, showing that the endoderm is following the ectoderm closely, and that a two-layered plate is really being formed even if obscured by the pressure of the two walls together (Fig. I). What the further development of such pieces would be, has not been definitely determined, but it is not likely that they close in to make a solid piece, for among the completely closed solid

pieces, there were none so large, unless the stem was thicker, or a ridge intervened.

Whatever the cause of closure may be, whether some kind of contraction or not, the fact can clearly be seen from the study of sections that cell-division has little or no part in the formation of the closing membrane; the new tube has practically the same number of cells as the open, part-cylinder. Mitotic figures are occasionally found, but not more than might have occurred in a normal tubularian, and in any case they were not frequent enough to indicate that mitosis is a factor in forming the closing membrane.

BRYN MAWR,
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